

09636499-101100

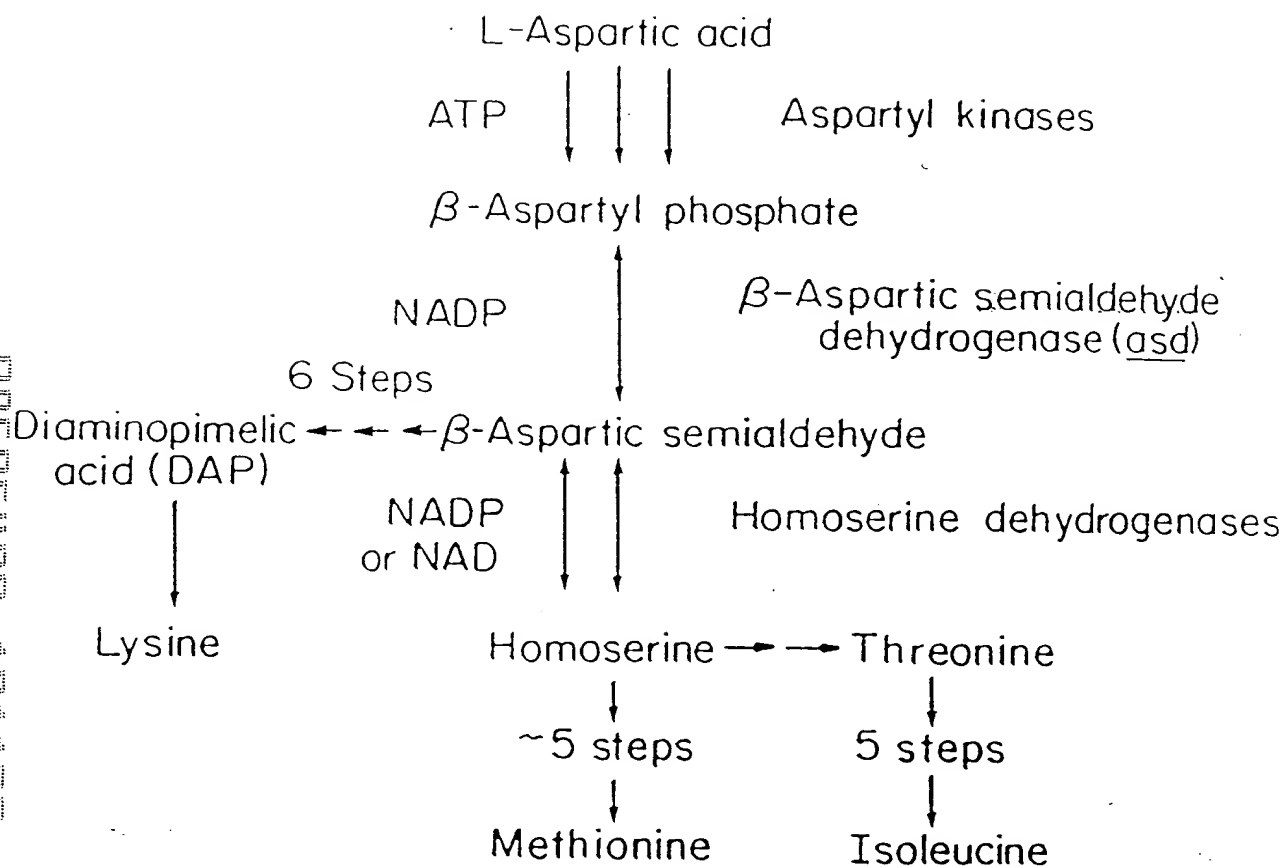


FIGURE 1

FIG. 2

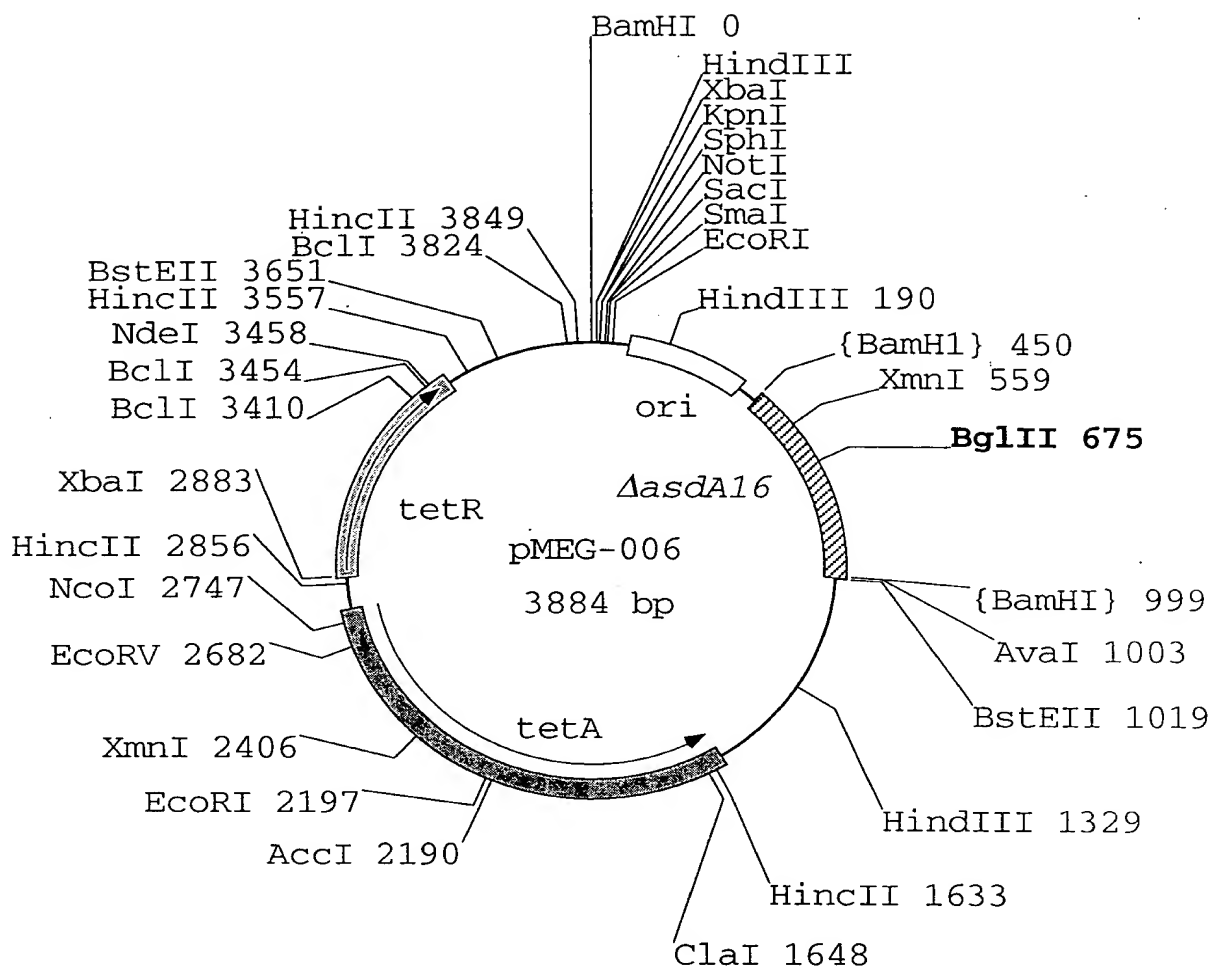
A

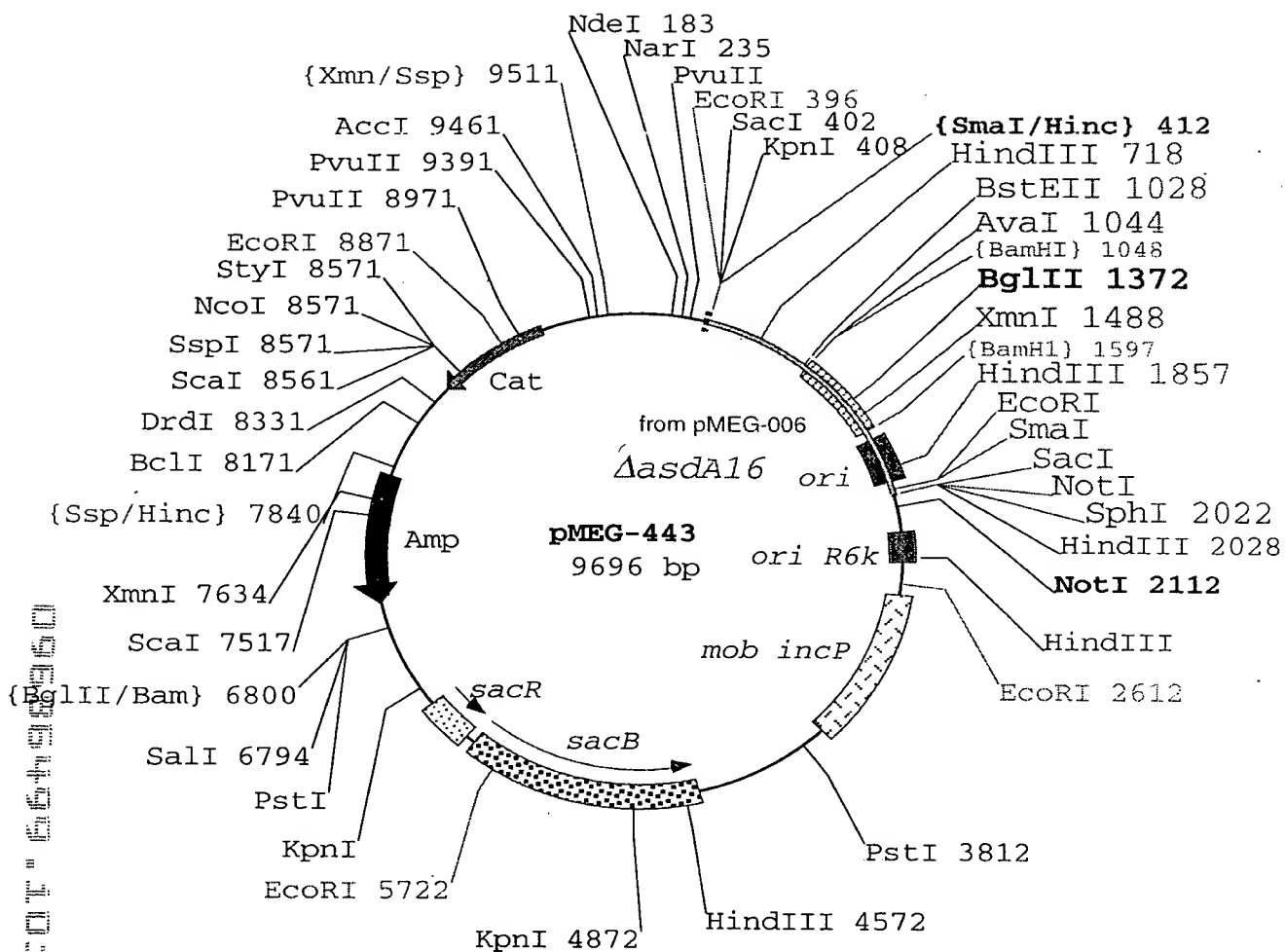
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121 taacgcaaat tccctgataa tcgccactgg actttctgct tgcgcggtaa ggcaggataa
181 gtcgcattac tgatggcttc gctatcattg attaatcca cttgcgactt tggctgcttt
241 ttgtatgggtg aaggatgcgc cacaggatac tggcgcgcac acacagcaca tctctttgca
301 ggaaaaaaac gctatgaaaa atgttggttt tatcggtgg cgcggaatgg tcggctctgt
361 tctcatgcaa cgcattgtag aggagcgcga ttctgacgct attcgccctg ttttcttttc
421 tacctcccag tttggacagg cggcgccac cttcgcgac acctccaccg gcacgtaca
481 ggacgctttt gatctggatg cgctaaaagc gctcgatata atcgtgacct gccaggcg
541 cgattatacc aacgaaattt atccaaagct gcgcgaaagc ggatggcagg gttactggat
601 tgatgcggct tctacgctgc gcatgaaaga tgatgccatt attattctcg acccggtaaa
661 ccaggacgtg attaccgacg gcctgaacaa tggcgtgaag acctttgtgg gcggttaactg
721 taccgttagc ctgatgttga tgcgctggg cggctctctt gcccataatc tcgttgactg
781 ggtatccgtc gcgacctatc aggcgcctc cggcgcgccg gcgcgcata tgcgcgagct
841 gttaaccag atgggtcagt tgatggcca tgcgcgcgac gaactggcga cgccgtcttc
901 cgcaattctt gatattgaac gcaaagttac ggcattgacc cgcagcggcg agctgccggg
961 tgataacttt ggcgtaccgc tggcggaag cctgatccc tggatcgaca aacagctcga
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1081 tgccctctgtg attccggttg atggtttgtg tgtgcgcgct gcgcgcgctg gctgtcacag
1141 ccaggcggtc accatcaagc tgaaaaaaga ggtatccatt ccgacggtg aagaactgct
1201 ggcggcacat aatccgtggg cgaaagtgg gccaacgat cgtgatata ctatgcgca
1261 attaaccccc gcggcggtga ccggcacgtt gactacgccc gttgggtcgtc tgcgtaagct
1321 gaacatgggg ccagagttct tgcggcgctt taccgtaggc gaccagttgt tatggggcgc
1381 gcccagccg ctgcgtcgaa tgctgcgca aggcgcgca ggtggcgtag tggctattgc agcgcttctc
1441 gggcctgcgt gtggttctgt aggcgggata aggcgcgca gcgcgccat ccggcgggga
1501 aatttgtgtt aaaccagggg tgcacgtca ccctttttt gcgtaataca ggagtaaacg
1561 cagatgtttc atttttatca ggagttaagc agagcattgg ctattcttta agggtagctt
1621 aatcccacgg gtattaagcc taacctgaag gtaggacgac gcagatagga tgcacagtgt
1681 gctgcgccgt tcagggtcaaa gaagtgtcac tacctgatgt tgaattggaa gatcc

B

MVKDAPQDTGAHTQHISLQEKNAKMNKVGFIGWRGMVGSVLMQRMVEERDFDAIRPVFFSTSQFGQAAPT
FGDTSTGTLQDAFDLDALKALDIIVTCQGGDYTNEIYPKLRESGWQGYWIDAASTLRMKDDAI IILDPV
NQDVITDGLNNGVKTFVGGNCTVSLMLSLGGLFAHNLDWVSVATYQAASGGGARHMRLLTQMGQLY
GHVADELATPSSAILDIERKVTALTRSGELPVDNFGVPLAGSLIPWIDKQLDNGQSREEWKQAETNKI
LNTASVIPVDGLCVRVGALRCHSQAFTIKLKKEVSIPTVEELLAHNPAKVPNDRDITMRELTPAAV
TGTLTTPVGRRLRKLNMGPFLSAFTVGDQLLWGAAEPLRRLRQLA

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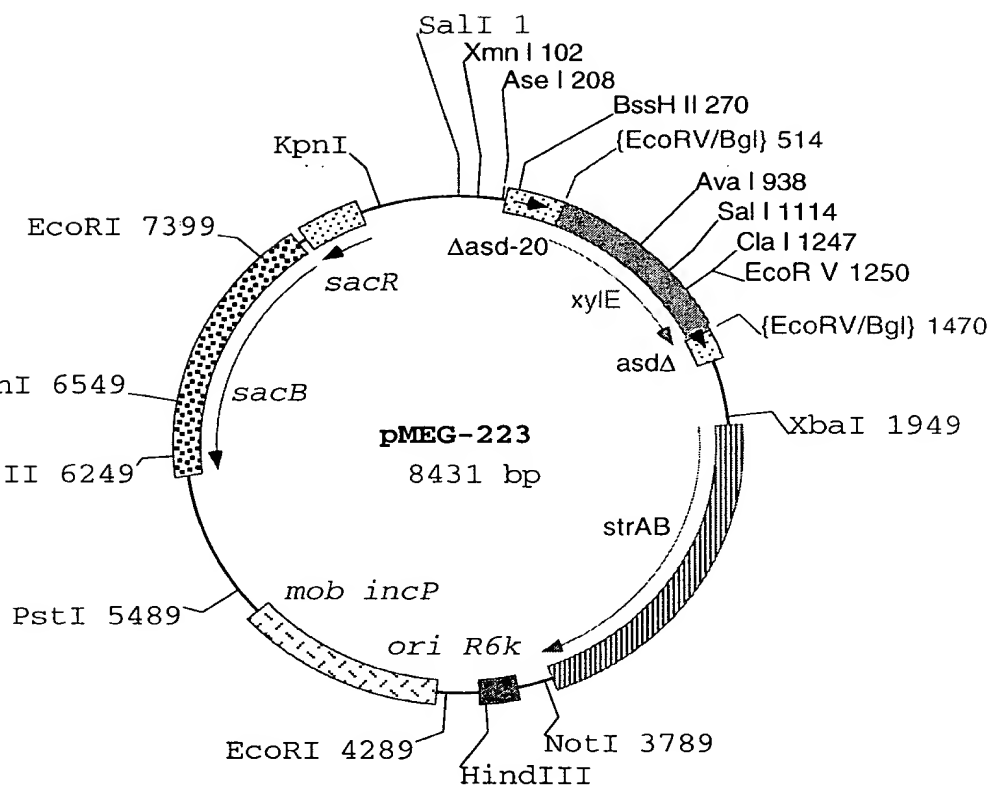


$$\{B_{\alpha_1}, B_{\alpha_2}, \dots, B_{\alpha_n}\} = \{I, I, \dots, I\}$$


pMEG-223
8431 bp

Genes and features: *sacR*, *sacB*, *xylE*, *asdΔ*, *strAB*, *ori R6k*, *mob incP*, $\Delta asd-20$.

Restriction enzyme sites (bp):
 SalI 1, XmnI 102, AseI 208, BssH II 270, {EcoRV/Bgl} 514, AvaI 938, SalI 1114, ClaI 1247, EcoRV 1250, {EcoRV/Bgl} 1470, XbaI 1949, NotI 3789, HindIII, EcoRI 4289, PstI 5489, HindIII 6249, KpnI 6549, EcoRI 7399, KpnI.



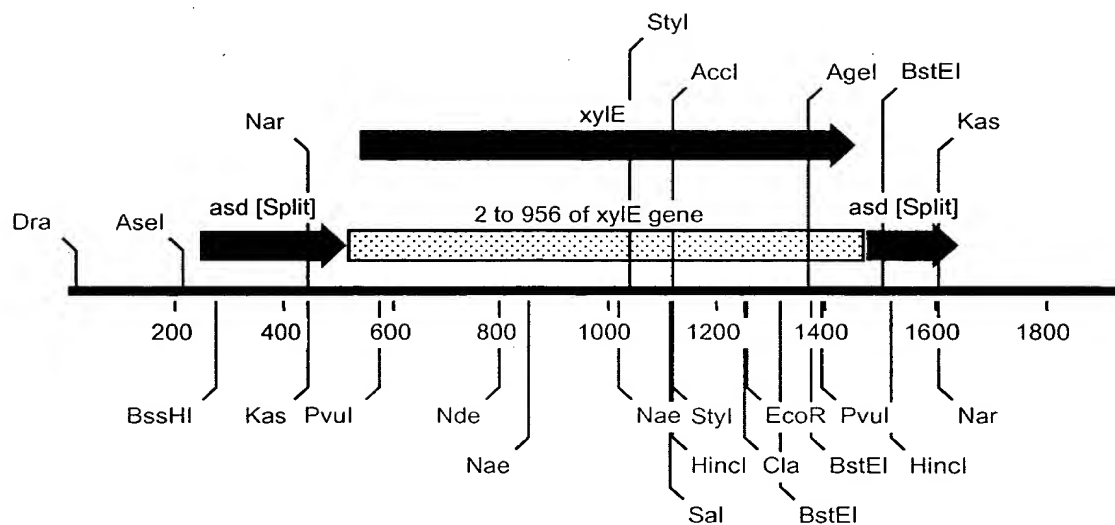


FIG. 6B

DraI

10 20 30 40 50 60
 GGATCTTCCCTAAATTTAAATATAAACAACGAATTATCTCCTTAACGTACGTTTTCGTTC
 70 80 90 100 110 120
 CATTGGCCCTCAAACCCCTAATTAGGATCAATAAAACAGCGACGGAAATGATTCCCTTCC
 130 140 150 160 170 180
 TAACGCAAATTCCCTGATAATCGCCACTGGACTTTCTGCTTGCGCGGTAAGGCAGGATAA

AseI

190 200 210 220 230 240
 GTCGCATTACTGATGGCTTCGCTATCATTGATTAATTTCACTTGCGACTTTGGCTGCTTT

BssHII

250 260 270 280
 TTGT ATG GTG AAG GAT GCG CCA CAG GAT ACT GGC GCG CAT ACA CAG
 Met Val Lys Asp Ala Pro Gln Asp Thr Gly Ala His Thr Gln
 ___a___a___a___a___a___ASD SPLIT]___a___a___a___a___a___
 290 300 310 320 330
 CAC ATC TCT TTG CAG GAA AAA AAC GCT ATG AAA AAT GTT GGT TTT
 His Ile Ser Leu Gln Glu Lys Asn Ala Met Lys Asn Val Gly Phe
 ___a___a___a___a___a___ASD [SPLIT]___a___a___a___a___a___
 340 350 360 370
 ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG
 Ile Gly Trp Arg Gly Met Val Gly Ser Val Leu Met Gln Arg Met
 ___a___a___a___a___a___ASD [SPLIT]___a___a___a___a___a___
 380 390 400 410 420
 GTA GAG GAG CGC GAT TTC GAC GCT ATT CGC CCT GTT TTC TTT TCT
 Val Glu Glu Arg Asp Phe Asp Ala Ile Arg Pro Val Phe Phe Ser
 ___a___a___a___a___a___ASD [SPLIT]___a___a___a___a___a___

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KasI

PvuII

1990年12月

[illegible]

AccI

11 11

1 1 1 1

EcoRV

Clal

BstEII

BstEII

AgeI

17422.doc

PvuII

1380 1390 1400 1410
GTG ACC TGG ACC ACC GAC CAG CTG GGC AAA GCC TTC TTT TAC CAC
Val Thr Trp Thr Thr Asp Gln Leu Gly Lys Ala Phe Phe Tyr His
___c___c___c___c___c___c___XYLE___c___c___c___c___c___c___

1420 1430 1440 1450
GAC CGC ATT CTC AAC GAA CGA TTC ATG ACC GTG CTG ACC
Asp Arg Ile Leu Asn Glu Arg Phe Met Thr Val Leu Thr
___c___c___c___c___c___c___XYLE___c___c___c___c___c___c___

BstEII

1460 1470 1480 1490 1500
TGATGGTCCGGAGATC ATC ACT ATG CGC GAA TTA ACC CCG GCG GCG GTG
Ile Thr Met Arg Glu Leu Thr Pro Ala Ala Val
___b___b___b___ASD [SPLIT]___b___b___b___b___

HincII

1510 1520 1530 1540 1550
ACC GGC ACG TTG ACT ACG CCG GTT GGT CGT CTG CGT AAG CTG AAC
Thr Gly Thr Leu Thr Thr Pro Val Gly Arg Leu Arg Lys Leu Asn
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

1560 1570 1580 1590
ATG GGC CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG
Met Gly Pro Glu Phe Leu Ser Ala Phe Thr Val Gly Asp Gln Leu
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

NarI

KasI

1600 1610 1620 1630 1640
TTA TGG GGC GCC GCG CCG CTG CGT CGA ATG CTG CGC CAG TTG
Leu Trp Gly Ala Ala Glu Pro Leu Arg Arg Met Leu Arg Gln Leu
___b___b___b___b___b___ASD [SPLIT]___b___b___b___b___b___b___

1650 1660 1670 1680
GCG TAGTGGCTATTGCAGCGCTTATCGGGCCTGCGTGTGG
Ala

1690 1700 1710 1720 1730 1740
TTCTGTAGGCCGGATAAGGCGCGTCAGCGCCGCCATCCGGCGGGGAAATTTGTGTAAAC

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

Nucleotide sequences of trc promoter/operator and MCS

MCS: *Nco*I *Eco*RI -----*Hind*III

pYA3098, pYA3148, pYA3332, pYA3333, pYA3334,

pYA3336, pYA3339, pYA3340, pYA3341, pYA3342

5' ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTC

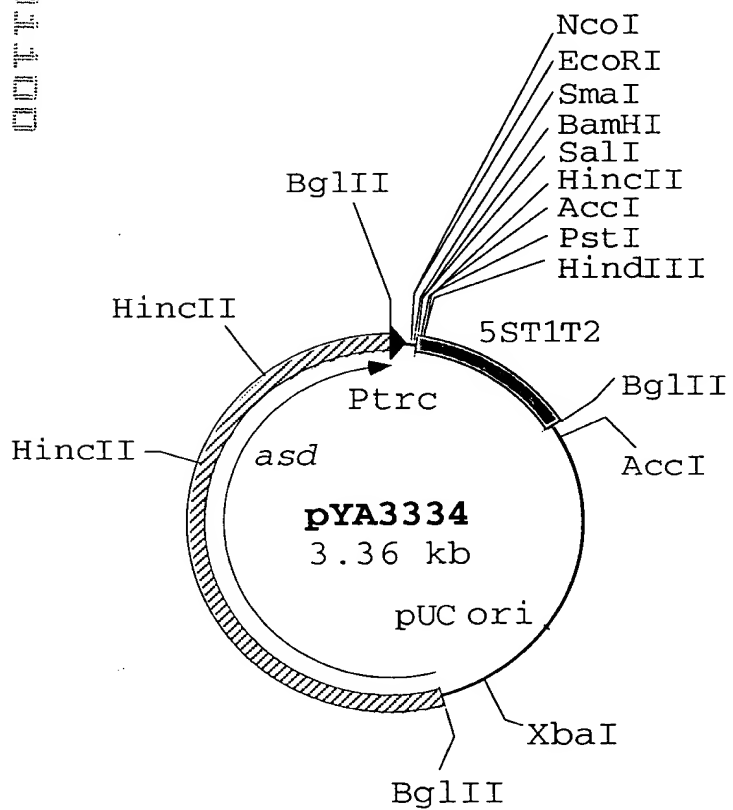
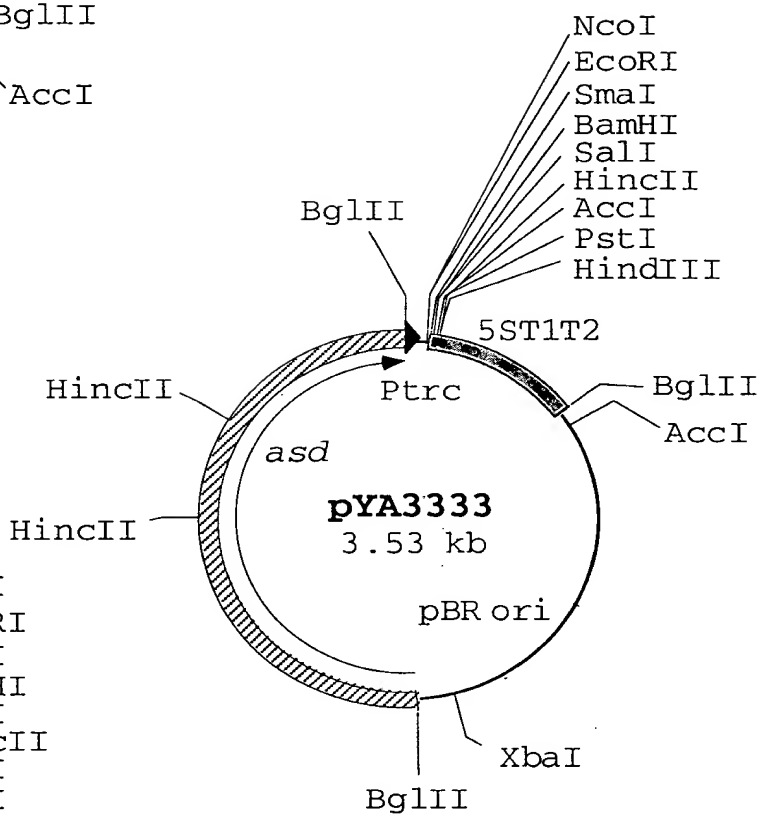
GTATAATGTGTGGAATTGTGAGCGGATAACAATTTACACAC

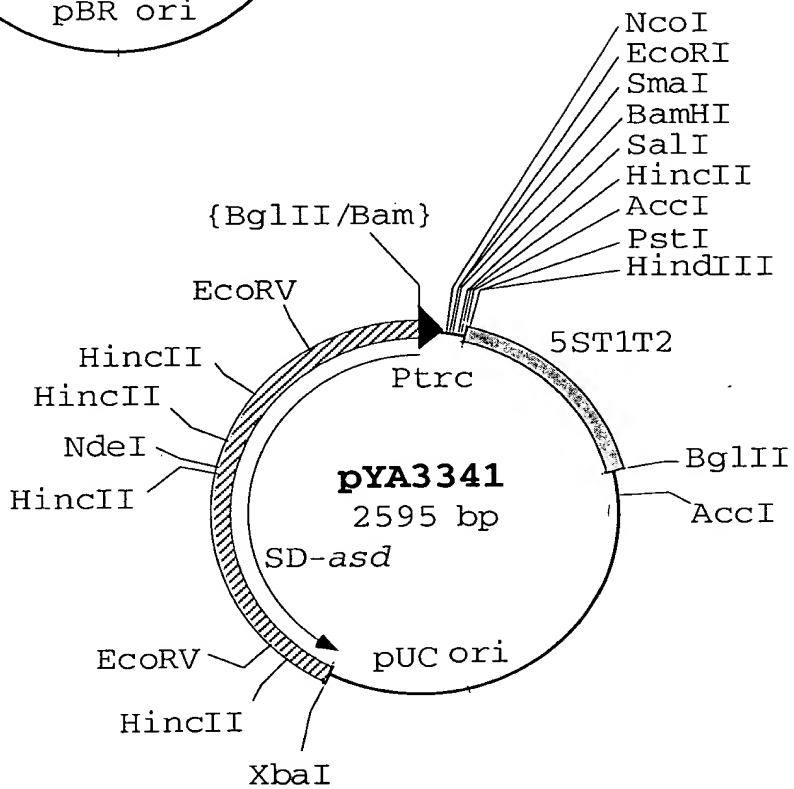
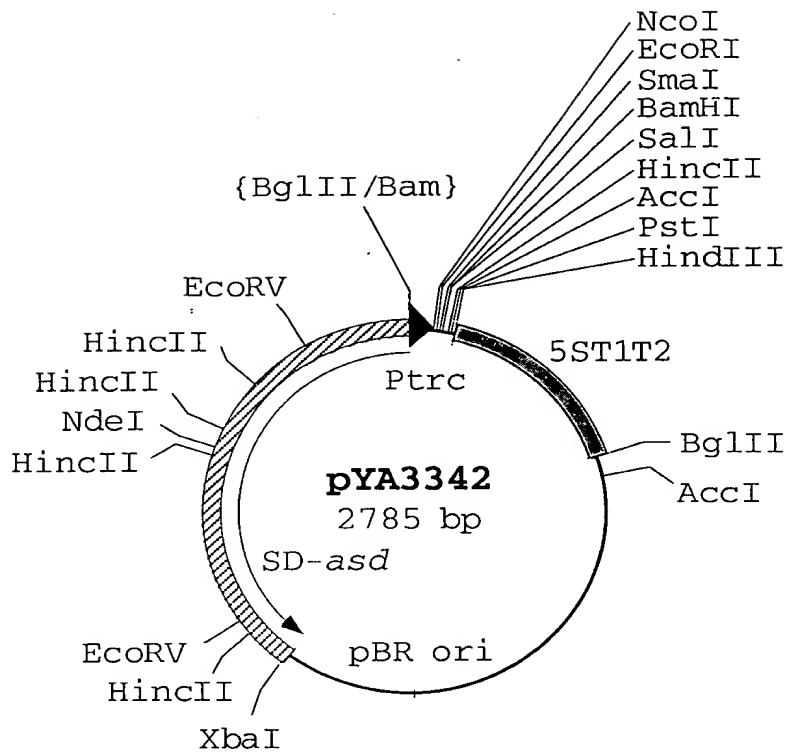
SD
*Nco*I
*Eco*RI
*Sma*I
 AGGAAACAGACC **ATG** GGA ATT CGC AAT TCC CGG GGA
Met Gly Ile Arg Asn Ser Arg Gly

*Bam*HI
*Sa*II
*Pst*I
*Hind*III
 TCC GTC GAC CTG CAG CCA AGC TCC CAA GCT T 3'
 Ser Val Asp Leu Gln Pro Ser Ser Gln Ala

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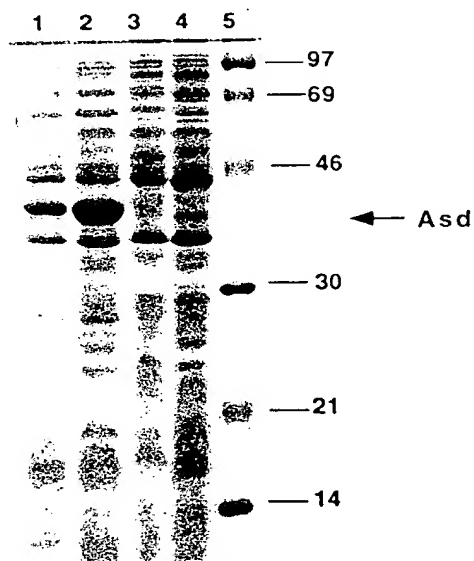
0963649





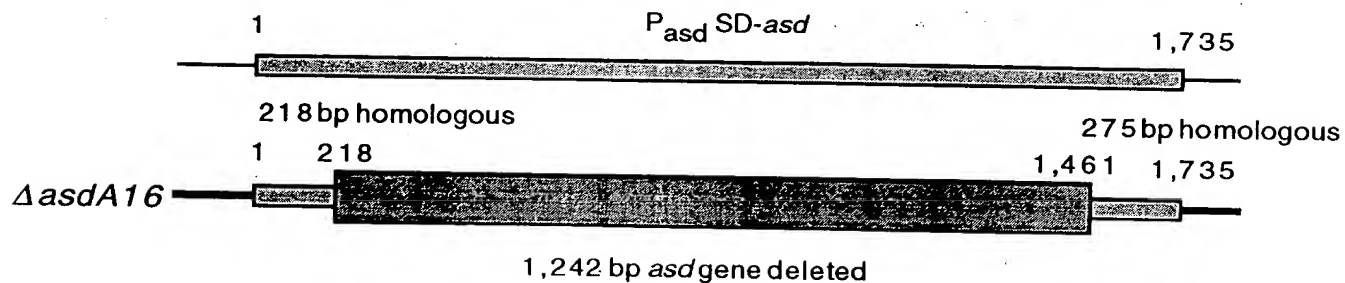
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Level of Asd synthesized in recombinant
S. typhimurium strains with different Asd⁺ plasmids



Cell lysates of *S. typhimurium* χ 4550 with pYA3333 (lane 1), pYA3334 (lane 2), pYA3342 (lane 3) and pYA3341 (lane 4). Lane 5 contains molecular weight markers. The arrow indicates Asd protein band.

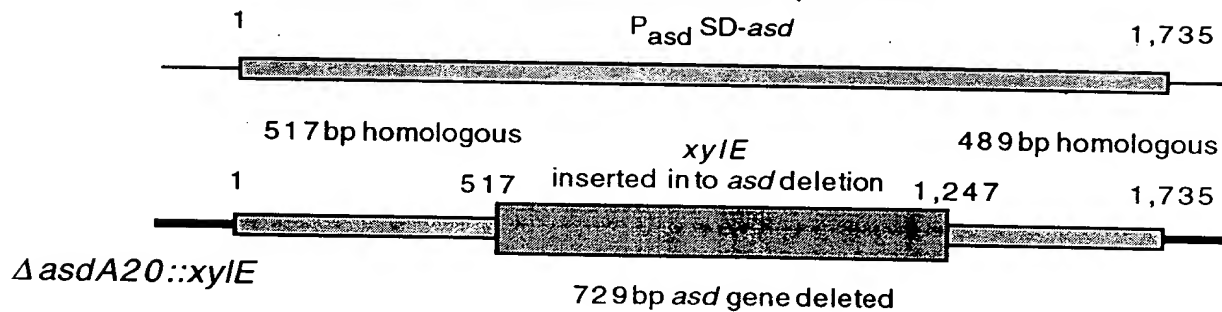
pYA3332, pYA3333 & pYA3334



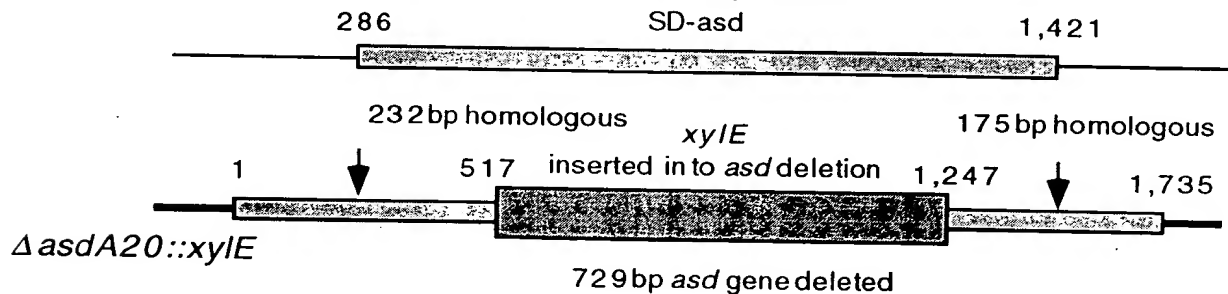
pYA3342 & pYA3341

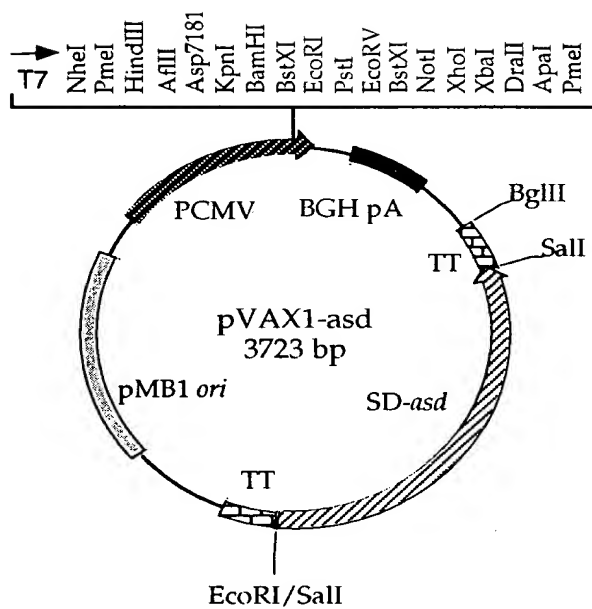


pYA3332, pYA3333 & pYA3334



pYA3342 & pYA3341





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